AD-A143 396 THE EFFECTS OF EDTA (ETHYLENEDIAMINETETRAACETIC ACID) AND SONICATION ON THE DISAGGREGATION OF ORAL BACTERIA (U) ARMY INST OF DENTAL RESEARCH WASHINGTON DC M B DAYOUB ET AL. 1984

UNCLASSIFIED F/G 6/5

1/1

NL

END

FILMED
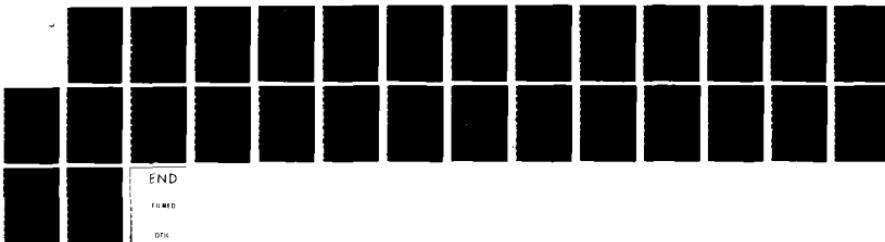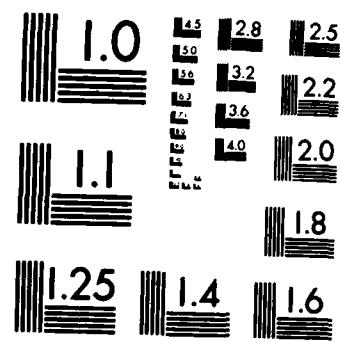
DTIC

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD-A143 396

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) The Effects of EDTA and Sonication on the Disaggregation of Oral Bacteria.	5. TYPE OF REPORT & PERIOD COVERED Manuscript for Publication
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) MICHAEL B. DAYOUB CHARLES E. HAWLEY JACK W. VINCENT	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Army Institute of Dental Research Walter Reed Army Medical Center Washington, DC 20012	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A; 3M161102BS10; DA: 365
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research & Development Command	12. REPORT DATE 1984
SGRD-RMS Fort Detrick, Maryland 21701	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)	15. SECURITY CLASS. (of this report) UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

This document has been approved for public release and sale; its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, If different from Report)

18. SUPPLEMENTARY NOTES

None

JUL 2 4 1984

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

SUMMARY: The dispersive effects of ethylenediaminetetraacetic acid (EDTA) and ultrasound on bacterial aggregates in an anaerobic environment were studied. Polled supragingival plaque samples were placed into reduced transport fluid (RTF) containing 1 mmol, 10 mmol, 100 mmol EDTA, or EDTA-free RTF for dispersion, and colony forming units (cfu) were determined after culture at 37C for 72 hours. Mean colony forming units were increased 42.7% and 54.4% respectively in 1 mmol and 10 mmol concentrations of EDTA and decreased when plaque was dispersed in 100 mmol concentrations of EDTA.

DTIC FILE COPY

Fusobacterium nucleatum was cultured in broth medium, placed into RTF containing no chelating agent, and sonicated for either 0, 5, 15, or 30 seconds. Sonication at the lowest power setting produced a statistically significant ($p < .01$) increase in CFU after 5 seconds. This trend did not continue when time of sonication was increased. The effect of sonication at medium or high power settings was variable, and factors affecting variability are discussed. EDTA and sonication may enhance the quantitation of oral microorganisms.

DISPOSITION FORM

For use of this form, see AR 340-15; the proponent agency is TAGO.

REFERENCE OR OFFICE SYMBOL	SUBJECT
SGRD-UDR-B	Request for Clearannce of Manuscript

TO   Cdr, USAIDR   FROM   COL M. B. Dayoub   DATE   28 March 1984   CMT 1

1. Request clearance for publication of the attached manuscript entitled "The Effects of EDTA and Sonification on the Disaggregation of Oral Bacteria" by M. B. Dayoub, C. E. Hawley, and J. W. Vincent.

2. If approved for publication, the journal of choice is Archives of Oral Biology.

1 Incl
as

MICHAEL B. DAYOUB
COL, DC
Microbiology Branch

SGRD-UDZ

TO   COL M. B. Dayoub   FROM   Cdr, USAIDR   DATE 29 Mar 84   CMT 2

Approved for publication.

THOMAS P. SWEENEY
COL, DC
Commanding

A-1

DA FORM 2496   PREVIOUS EDITIONS WILL BE USED   U S G P O 1982 - 386 545
AUG 80

THE EFFECTS OF EDTA AND SONICATION ON THE

DISAGGREGATION OF ORAL BACTERIA.

RUNNING TITLE: BACTERIAL PLAQUE DISAGGREGATION

MICHAEL B. DAYOUB
CHARLES E. HAWLEY*
JACK W. VINCENT
Division of Research Sciences
U. S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D. C. 20307

*   Department of Periodontics
    Baltimore College of Dental Surgery
    University of Maryland at Baltimore
    Baltimore, Maryland

84 07 02 014

THE EFFECTS OF EDTA AND SONICATION ON THE
DISAGGREGATION OF ORAL BACTERIA.

RUNNING TITLE: BACTERIAL PLAQUE DISAGGREGATION

SUMMARY

The dispersive effects of ethylenediaminetetraacetic acid (EDTA) and ultrasound on bacterial aggregates in an anaerobic environment were studied. Pooled supragingival plaque samples were placed into reduced transport fluid (RTF) containing 1 mmol, 10 mmol, 100 mmol EDTA, or EDTA-free RTF for dispersion, and colony forming units (cfu) were determined after culture at 37C for 72 hours. Mean colony forming units were increased 42.7% and 54.4% respectively in 1 mmol and 10 mmol concentrations of EDTA and decreased when plaque was dispersed in 100mmol concentrations of EDTA. *Fusobacterium nucleatum* was cultured in broth medium, placed into RTF containing no chelating agent, and sonicated for either 0, 5, 15, or 30 seconds. Sonication at the lowest power setting produced a statistically significant ($p < .01$) increase in CFU after 5 seconds. This trend did not continue when time of sonication was increased. The effect of sonication at medium or high power settings was variable, and factors affecting variability are discussed. EDTA and sonication may enhance the quantitation of oral microorganisms.

INTRODUCTION

The microbiota of dental plaque has been classified and quantitated by a variety of methods (Rosebury, MacDonald and Clark 1950; Loesche, Hockett and Syed 1972; Loesche and Syed 1973; Williams; Pantalone and Sherris 1976; Weiner, et al 1978). In all instances, one essential step was the disaggregation and dispersion of plaque samples. Because of the tendency of plaque bacteria to adhere to each other (Gibbons and Nygard 1970), to red blood cells (Falkler and Hawley 1975), and to other eukaryotic cells (Falkler, Smoot and Mongiello 1982) the ability to isolate pure cultures for identification and the accurate quantitation of some bacterial species may be compromised. In addition, the use of extreme means of dispersal may destroy certain bacteria, such as certain gram-negative species, thus leading to a gross underestimation of their numbers in a given plaque sample. It is thus evident that the precise identification and quantitation of the bacteria in dental plaque depends upon (1) the degree of microbial dispersion achieved prior to culture and, (2) the effect of dispersion techniques on the cultivability of these organisms in vitro.

In the past, the most common dispersive technique has involved the mechanical agitation of plaque samples in a suspending medium. Other methods have provided dispersion

through the use of a hypodermic syringe (Williams et al. 1976) or

a blender (Loesche and Syed 1973). Recent studies have shown the

value of the divalent cation chelator, ethylenediaminetetraacetic

acid (EDTA) (Loesche et al. 1972) or sonic oscillation (Loesche

and Syed 1973 and Robrish et al 1976).

The purpose of this study was to demonstrate the effects of

various concentrations of EDTA and modifications of sonic tech-

niques on the dispersion and cultivability of plaque bacteria.

MATERIALS AND METHODS

EDTA As A Bacterial Disaggregant

Figure 1 shows the processing scheme for plaque samples with

which chelator-associated dispersion was evaluated.   Using a

curette, supragingival dental plaque was obtained from six

patients and placed immediately into chelator-free RTF (Loesche

et al 1972).   The samples were pooled and placed into an

anaerobic chamber (Coy Laboratory Products Inc., Ann Arbor,

Michigan) containing 10% H_2, 5% CO_2, and 85% nitrogen (Aranki et

al 1969).   Serial tenfold dilutions of the pooled plaque were

then carried out in unmodified RTF or in RTF which contained

either 1 mmol, 10 mmol, or 100 mmol EDTA.   (Sigma Chemical Co.,

St. Louis, Missouri).   Aliquots (0.1 ml) of each dilution were

spread in triplicate on prereduced Schaedler agar plates that

were supplemented with 50 ml/L defibrinated sheep blood (Flow

Laboratories, McLean, Virginia) and 0.5 g/ml menadione.   The

plates were cultured for 72 hours anaerobically (<-140 mv) at 37C

and the number of colony forming units (CFU) per aliquot was determined for aliquots showing 30 to 300 colonies per plate.

Sonication As A Bacterial Disaggregant

The effect of sonication on bacterial disaggregation and viability was examined in the same controlled anaerobic environment. The gram-negative anaerobic bacterium, Fusobacterium nucleatum (ATCC 23726) (American Type Culture Collection, Rockville, Maryland) an organism shown to be sensitive to the effects of sonication (Williams and Eickenberg 1952; Robrish et al 1976 and Aranki et al 1969) was cultured in Brewer thioglycollate medium (Becton Dickenson and Co., Cockeysville, Maryland) for 48 hours at 37C. This organism was utilized based on known difficulty in dispersion and its tendency to bind galactose sensitive receptors on eukaryotic cells (Mongiello and Falkler 1979). Aliquots (2.0ml) of the broth culture were placed into sterile 12x75 mm serologic test tubes (Falcon Plastics, Oxnard, California) for dispersion testing.

A sonic oscillator (Kontes Cell Disruptor, Vineland, New Jersey) with a 4 and 1/2 inch titanium magnetostrictive probe was used at low (#1), medium (#4), and high (#8) power settings. The power output for these settings was given by the manufacturer as 292, 541, and 1200 Watts per sq.inch, respectively, and the tip frequency for this unit was rated at 23,500 cycles per second with a displacement of 100 u meters double amplitude. Two sets of trials were performed. In the first experiment, sonication

was performed for 0, 5, 15, and 30 seconds in three trials at each of the designated power settings. In a second experiment sonication was performed for 0, 5, and 15 seconds, in five trials, using only the lowest power setting. After sonication, 1.0 ml aliquots of each test were subjected to tenfold dilutions, spread on duplicate Schaedler supplemented agar plates, and incubated at 37°C in the anaerobic chamber. After incubation of 72 hours, viable counts were determined of dilutions demonstrating 30-300 CFU per plate.

Throughout the study all sampling or dilution procedures were preceded by a 30 second vortex (Vortex-Genie, Scientific Industries, Queens Village, New York) in order to obtain a uniform distribution of the bacterium in both control and experimental samples.

Statistics

Statistical methods consisted of the analysis of variance of plate counts in square root transform as usually applied to stabilize the variance in Poisson variates. Treatment-comparisons were made as sensitive as possible by utilizing the principle of blocking to remove trial-to-trial variation. The principle is analogous to the paired comparison concept in the t-test, generalized in the analysis of variance.

RESULTS

Three concentrations of EDTA in RTF were used to test the effect of EDTA on the disaggregation of plaque bacteria. The

resulting CFU were compared to CFU obtained from RTF which contained no EDTA (Table 1). Mean CFU were averaged for all trials and are shown graphically in log transform (Figure 2). Dispersion by vortex was used in all samples during dilution. The mean CFU from six plaque samples increased 42.7% (185.2) and 54.4% (204.3) over nonchelated controls (129.8) in 1 mmol and 10 mmol concentrations of EDTA, respectively, and CFU decreased 14.1% (111.5) below controls when plaque was dispersed in 100 mmol EDTA. A statistical analysis of variance met the $p < .08$ level of probability for all groups. When the paired t-test was used, mean CFU for 10 mmol EDTA and the non-chelated control group showed differences which were statistically significant ($p < .02$). The differences between the control without EDTA and experimentals with 1 mmol or 100 mmol EDTA were not statistically significant ($p > .10$).

In the first sonication experiment, the combined effect of instrument power setting and sonication time was determined for the number of cultivable F. nucleatum in three trials (Figure 3). Mean CFU of triplicate cultures were transformed to square roots and tested for significance. Analysis of variance showed no significant differences between mean CFU produced with 5, 15, or 30 seconds of sonication or between mean CFU obtained from each of the three instrument power settings at any sonification time. The difference between the apparent increased dispersion observed at low power settings after five seconds and the decreased dis-

persion noted at the two other power settings (Figure 3) was tested statistically and found to be significant at the $p < .05$ level of probability.

In the second sonication experiment, five dispersion trials were performed at the low power setting only. CFU of triplicate plates were again transformed to square roots and analyzed in a random block design by the analysis of variance. As shown in Figure 4, five second sonic disaggregation of F. nucleatum was effective in producing a 66% increase in CFU (242.7) over the number of CFU obtained without sonication (160.3). Again, dispersion by vortex was used in all samples during dilution. These differences were statistically significant at the $p < .01$ level of probability. Sonication at the low power setting between 5 and 15 seconds did not result in statistically significant changes in CFU.

Because of similarities in the dispersion profiles at the low power settings, the data obtained at 0, 5, 15 seconds during the first experiment (n=3) (Figure 3) was combined with parallel data obtained during the second experiment (n=5) (Figure 4). Figure 5 graphically shows the overall 73.3% increase in recovery of viable F. nucleatum after a five second sonication ($p < .01$). Again, the significance of differences in CFU obtained between 5 and 15 second sonication at the low power setting were statistically indistinguishable.

DISCUSSION

A previous study (Loesche et al. 1972) showed that the use of a 1 mmol solution of EDTA resulted in a twofold increase in the microscopic count of plaque bacteria. In the present study, CFU counts from supragingival plaque were used to evaluate the disaggregating effects of EDTA. At 1 mmol and 10 mmol concentrations of EDTA, there appeared to be increased disaggregation of plaque bacteria. A one mmol solution of EDTA showed a mean increase of 42.7% in CFU over controls without EDTA, and 10 mmol concentrations of EDTA yielded CFU counts 57.4% higher than the same controls. At 100 mmol concentrations, CFU counts were decreased below control levels, which suggested that EDTA had a bactericidal, or growth inhibiting effect, on plaque organisms. This growth inhibiting effect may have been due to a chelator associated disruption of external cell envelope components such as the reported alterations in lipopolysaccharide structure of gram-negative bacteria (Birdsell and Ceta-Robles, 1967; Reynolds and Pruul, 1971) or the reported disassociation of cell wall associated essential enzyme systems (Schlesinger, 1968; Torriani, 1968). The magnitude of any detrimental effect of 1 mmol and 10 mmol EDTA on CFU could not be evaluated in this investigation since it was probably obscured by the significant increases in plaque CFU produced at these concentrations of EDTA. However, the marked difference between the 200% increase of microorganisms previously observed microscopically (Loesche et al, 1973), and the 42% to 54% increase in cultivable bacteria reported here may

have been due, in part, to an EDTA associated effect upon microbial viability. Therefore, these results probably reflect the effect of both bacterial aggregate dispersion in 1 mmol and 10 mmol EDTA and suspected variations in microbial physiology. The results presented here do suggest that 10 mmol EDTA may be useful in enhancing the disaggregation of dental plaque samples, and the benefits of enhanced cell dispersion at this concentration probably outweigh any potential negative effect of 10 mmol EDTA on bacterial growth.

Variables involved in sonication procedures are frequently unrecognized or ignored, and they should be given greater consideration. Investigations have shown that ultrasonic disruption of bacteria may be affected by the volume and viscosity of the sample, the size and rigidity of the container, the area of air interface, and the ultrasonic probe depth within the medium. Also, the use of ultrasonic probes with high end velocities, or the use of excessively high power settings, in relation to the volume sonicated, can result in fluid turbulence (cavitation unloading) (Hughes, 1961).

In this study, every attempt was made to hold these factors constant, except that a consistent probe depth could not always be maintained. It is believed that the turbulence caused by cavitation unloading produced the rapid changes in surface levels and in relative probe depths observed during our high energy sonication tests. The production of turbulence was particularly

evident when sonicating at medium and high power settings, and was observed to be dependent upon the level of the probe within the bacterial suspension.

The effects of sonication on CFU depended upon the duration and intensity of sonic energy employed. The tendency of CFU to decrease precipitously following a five second exposure to the medium or high power setting (Figure 3) may have been due to the lethal effects of sonic energy on previously unaggregated test organisms. As sonication time increased to 15 and 30 seconds, the same high power settings were noted to generate cavitation unloading in the samples. It is thought that the rapid degassing and turbulence of the fluid medium may have neutralized the expected bactericidal effect of sonic energy and thereby affected the observed increase in CFU after five seconds at both medium and high power settings. Cavitation unloading was not evident during trials at the low setting, and there was a marked (66%) increase in CFU observed after five seconds of sonication (Figure 4). Sonication from 5 to 15 seconds at the low power setting did not significantly affect changes in bacterial counts (Figure 4). At 30 seconds the combined effect of low energy sonication and duration of exposure on bacterial viability produced a more apparent reduction in CFU (Figure 3).

It has been demonstrated that differences occur in sensitivity of organisms to lethal damage during sonic treatment, and that susceptibility is greater with gram-negative organisms,

including F. nucleatum (Robrish, et al, 1976). Others have reported that less than 30 seconds sonication may adversely affect cultivable numbers of oral bacteria (Torriani, 1968: Spiegel, 1978). Under the conditions of this study, it has been shown that counts of cultivable gram-negative organisms may increase after five seconds of low energy sonic treatment. These results also indicate that the previously reported lethal effect on F. nucleatum was not observed after 15 seconds of exposure to low energy sonic oscillation. This study, therefore, suggests that many of the previously reported differences regarding the lethal consequences of sonic energy may reflect manifestations of any number of the variables listed earlier (volume, viscosity, and air/fluid interface). These data also stress the importance of duration and intensity of sonic energy to the cultivability of F. nucleatum

In conclusion, we have shown that the divalent cation chelator, EDTA, can be employed to significantly increase the number of cultivable plaque bacteria. Enhanced dispersion in the presence of EDTA could be important to investigations where it is essential to identify and/or quantitate the plaque organisms related to oral disease processes. In addition, it was apparent that low energy sonication from 5 to 15 seconds can be used to produce significant dispersion of normally sonication-sensitive gram-negative organisms such as F. nucleatum. Studies which will explore the combined effects of EDTA and low energy sonication

are being planned.

* * * * * *

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

LEGENDS

Figure 1. Method of Processing Plaque Samples in Various Chela:or
Concentations to Test for Bacterial Dispersion.

Figure 2. The Effect of EDTA Concentration on Bacterial Disaggregation
(Mean ± Standard Error).

Figure 3. The Effect of Time and Sonicator Power Setting on Cultivable
Numbers of Fusobacterium nucleatum.

Figure 4. The Effect of Sonication at Low Power Setting on Cultivable
Numbers of Fusobacterium nucleatum. Mean ± Standard Error.

Figure 5. The Effect of Time at Low Sonicator Power Setting on Cultivable
Numbers of Fusobacterium nucleatum. All (n=8) Trials. Mean ±
Standard Error.

METHOD

SUPRAGINGIVAL PLAGUE FROM SIX PATIENTS

REDUCED TRANSPORT FLUID WITHOUT EDTA

DISPERSED
BY VORTEX
FOR 30
SECONDS

REDUCED TRANSPORT FLUID

CONTROL   Immol   IOmmol   IOOmmol

DISPERSED
BY VORTEX
FOR 30
SECONDS

MSSB*   MSSB   MSSB   MSSB
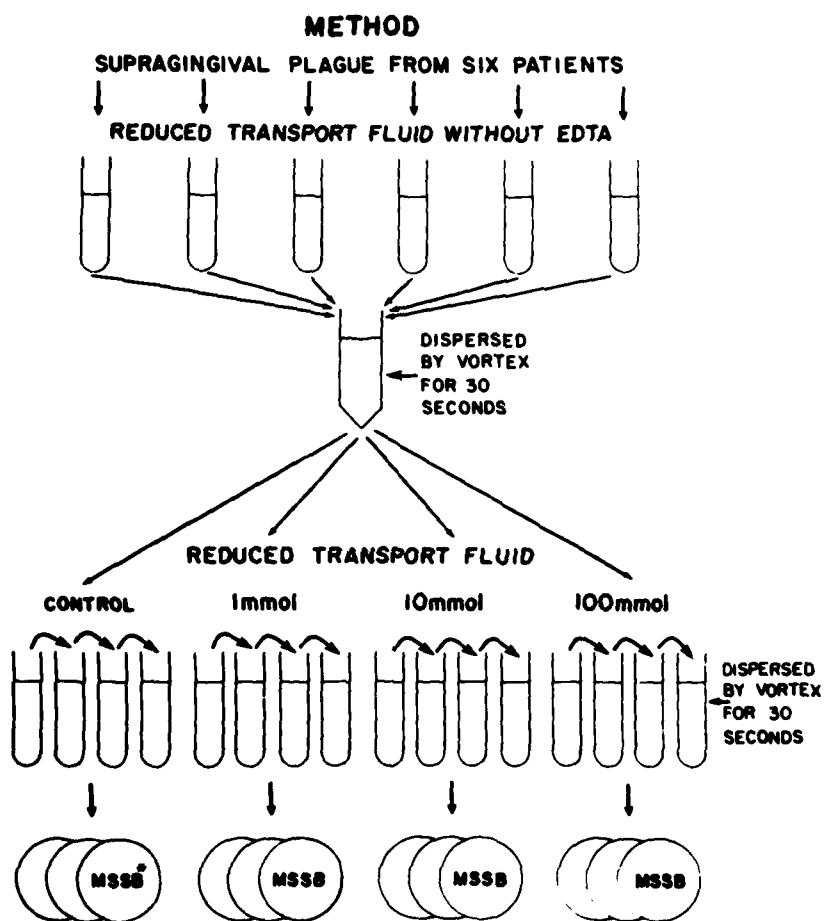
*MENADIONE SUPPLEMENTED SHAEDLER BLOOD AGAR PLATES

Fig 1

Fig 2

MEAN CFU F. nucleatum / 0.1 ml
TRANSPORT FLUID

SONICATION (SECONDS)

POWER SETTING #1

POWER SETTING #4

POWER SETTING #8
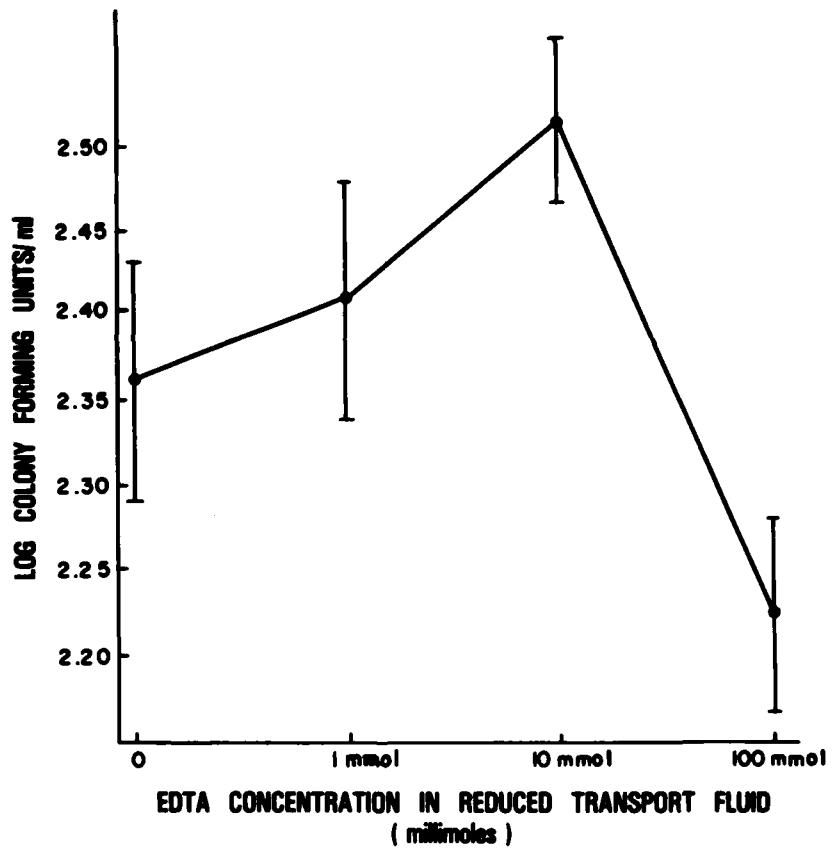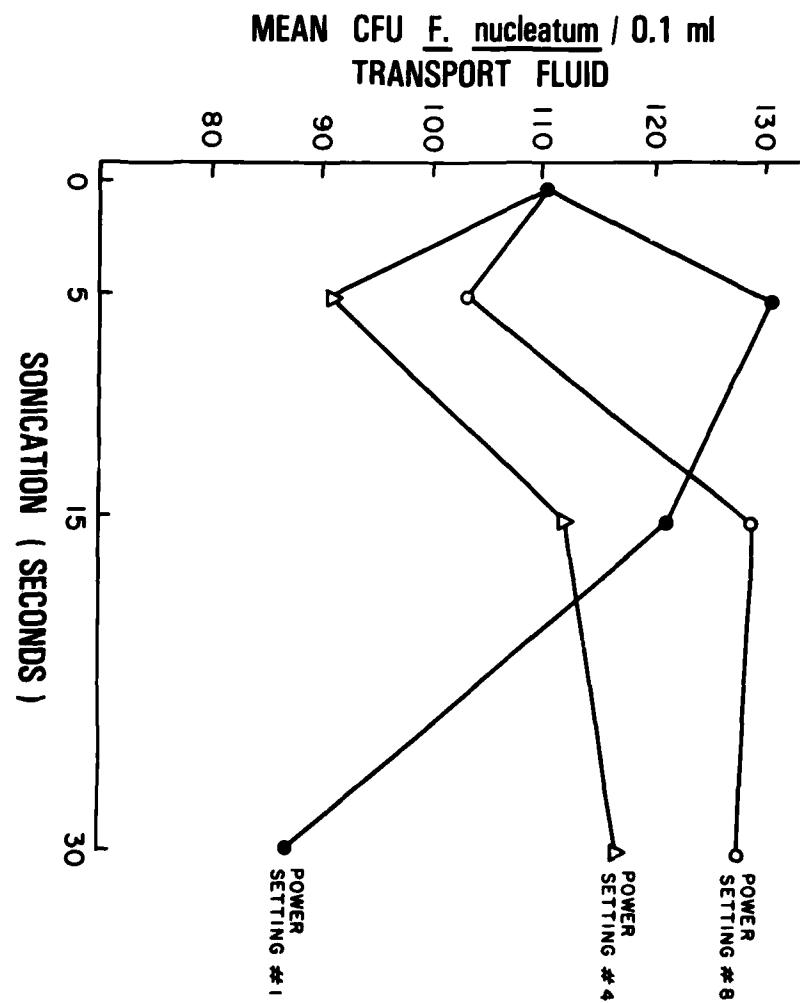
Fig 3
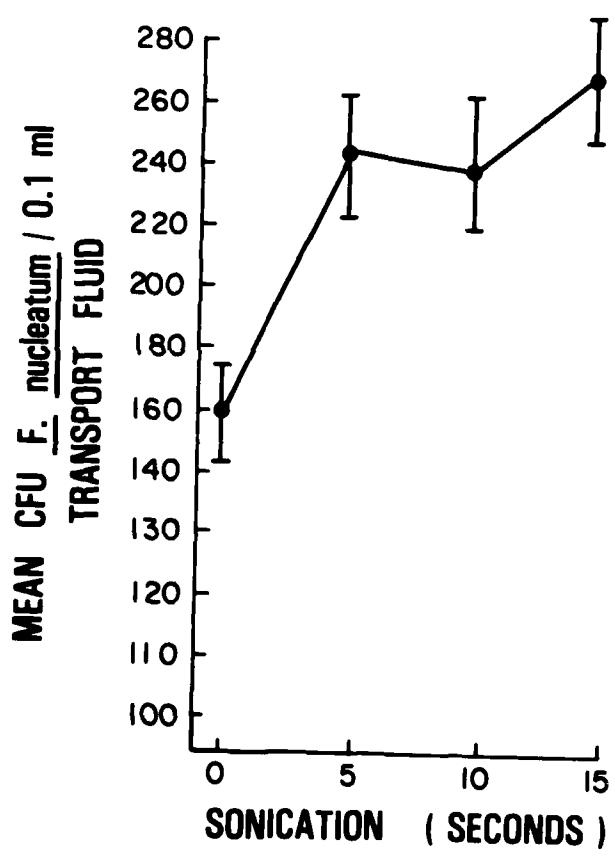
Fig 4
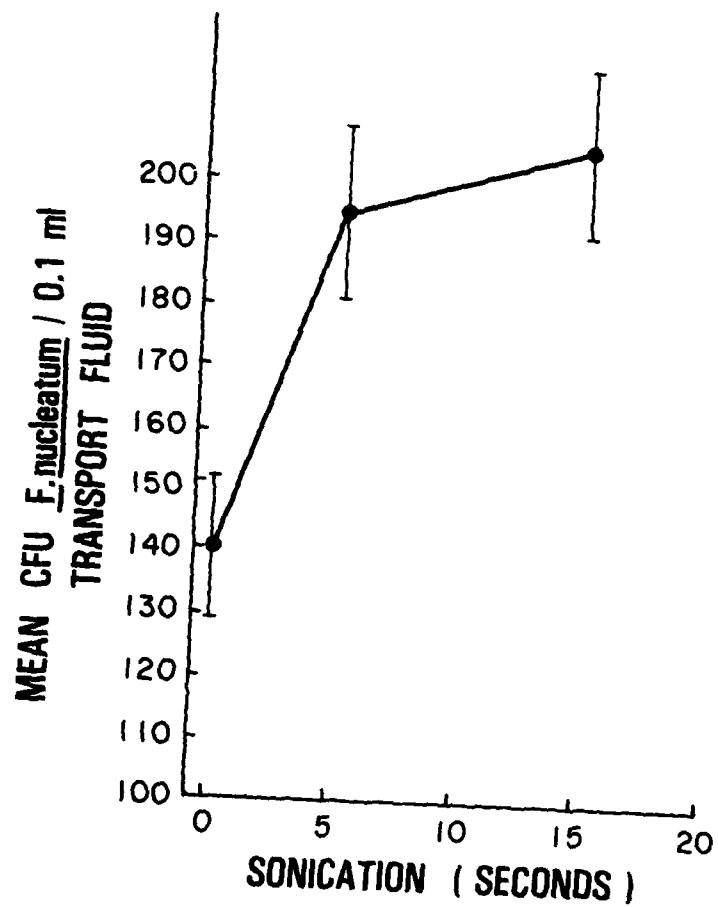
Fig 5

TABLE 1. Mean counts of colony Forming Units in 0.1 ml

   Aliquots From Supragingival Plaque Treated With EDTA.

Trial	Control	1 mmol EDTA	10 mmol EDTA	100 mmol EDTA
1	235	411	316	205
2	198	300	243	111
3	72	50	156	31
4	106	152	253	35
5	133	163	204	251
6	35	35	54	36
Mean	129.8	185.2	204.3	111.5
Standard Error	30.9	59.6	37.0	39.0

REFERENCES

Aranki, A., Syed, S.A., Kenney, C.B. and Freter, R. (1969) Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. 17, 568-576.

Birdsell, D.C. and Ceta-Robles, E.H. (1967) Production and ultrastructure of lysozyme and ethylene diamine tetraacetate-lysozyme spheroplasts of Escherichia coli. J. Bacteriol. 93,427-437.

Boucher, R.M.G. Advances in sterilization techniques. (1972) Am. J. Hosp. Pharm. 29, 660-672.

Falkler, W.A. Jr., and Hawley, C.E. (1977) Hemagglutinating activity of Fusobacterium nucleatum. Infect. Immun. 15,230-238.

Falkler, W.A. Jr., Smoot, C.N. and Mongiello, J.R. (1982) Attachment of cell fragments of Fusobacterium nucleatum to oral epithelial cells, gingival fibroblasts and white blood cells. Arch. Oral Biol. 27, 553-559.

Gibbons, R.J. and Nygard, M. (1970) Interbacterial aggregation of plaque bacteria. Arch. Oral Biol. 15, 1397-1400.

Gibbons, R.J. and VanHoute, J. (1973) On the formation of dental plaque. J. Periodontol. 44, 347-360.

Hughes, D.E. (1961) The disintegration of bacteria and other microorganisms by the M.S.E.-Mullard ultrasonic disintegrator.

Journal of Biochemical and Microbiological Technology and Engineering. 3, 405-433.

Leadbetter, E.R. and Holt, S.C. (1974) Influence of sonication on cultivable microbiota of dental plaque. J. Dent. Res. 53 (Special Issue),106.

Loesche, W.J., Hockett, R.N. and Syed, S.A. (1972) The predominant cultivable flora of tooth surface plaque removed from institutionalized subjects. Arch. Oral Biol. 17, 1311-1325.

Loesche, W.J. and Syed, S.A. (1973) The predominant cultivable flora of carious plaque and carious dentin. Caries Res. 7, 201-216.

Mongiello, J.R. and Falkler, W.A.,Jr. (1979) Sugar inhibition of oral Fusobacterium nucleatum hemagglutination and cell binding. Arch. Oral Bio. 24, 539-545.

Reynolds, B.L. and Pruul, H. (1971) Protective role of smooth lipopolysaccharide in serum bacterial reaction. Infect. Immun. 4, 764-771.

Robrish, S.B., Grove, R.S., Bernstein, P.T., Marucha, P.T., Socransky, S.S., and Amdur, B. (1976) Effect of sonic treatment on pure cultures and aggregates of bacteria. J. Clin. Microbiol. 3, 474-479.

Rosebury, T., MacDonald, J.B. and Clark, A.R. (1950) A bacteriologic survey of gingival scrapings from periodontal infections by direct examination, guinea pig inoculation, and

anaerobic cultivation. J Dent Res. 29, 718-731.

Schlesinger, M.J. (1968) Secretion of alkaline phosphatase subunits by spheroplasts of Escherichia coli. J. Bacteriol. 96, 727-733.

Spiegel, C. A. (1978) Ph.D. Thesis: The predominant cultivable microflora of plaque from healthy and diseased gingival sulci with emphasis on the black pigmented Bacteroides and the anaerobic gram positive cocci. University of Maryland.

Torriani, A. (1968) Alkaline phosphatase subunits and their dimerization in vivo. J. Bacteriol. 96, 1200-1207.

Weiner, M., Grienko, V., Kaplan, B. and Newman, M.C.(1978) A simplified technique for processing plaque samples. J. Dent. Res. 57, (Special Issue A):243.

Williams, B.L., Pantalone, R.M. and Sherris, J.C. (1976) Subgingival microflora and periodontitis. J. Periodont. Res. 11, 1-18.

Williams, N.B. and Eickenberg, C.F.(1952) Effects of sonic vibration on the numbers of microorganisms cultivable from human saliva. J. Dent. Res. 31, 428-439.

Williams, R.C. and Gibbons, R.J. (1975) Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect. Immun. 11, 711-718.

for Reprints:

COL Michael B. Dayoub, DC

Division of Research Sciences

U. S. Army Institute of Dental Research

Walter Reed Army Medical Center

Washington, DC   20307   U.S.A.